

Mapping Lac_Y -

201

May 21, 1948.

W-67 × Y64 Lac_Y-V,^S × Lac₂-V,R.

Among 28 plates carrying ca 150 good-sized colonies each, only 7 + colonies were noted (2 missing). Ca 1/400 + : - . Score +'s for phage resistance

Lac + (only 3 rapid +) ALL R.

Lac - :	R	S
10	0	
20	0	
18	0	
	48.	0

Sensitives are again missing.

3 hypotheses:

- ① Lac_Y- is a lethal in sexual progeny
- ② Lac_Y- is linked to a "lethal" which may be a nutritional requirement
- ③ Lac_Y,+ are not produced in these crosses due to chromosome aberrations or a related phenomenon.

- ① Check nutrition of W-67
- ② Cross W-67 and Y64 on glucose medium
- ③ If an "inhibitor" what are the limits of its action.

Lac_3 ; Lac_4

202

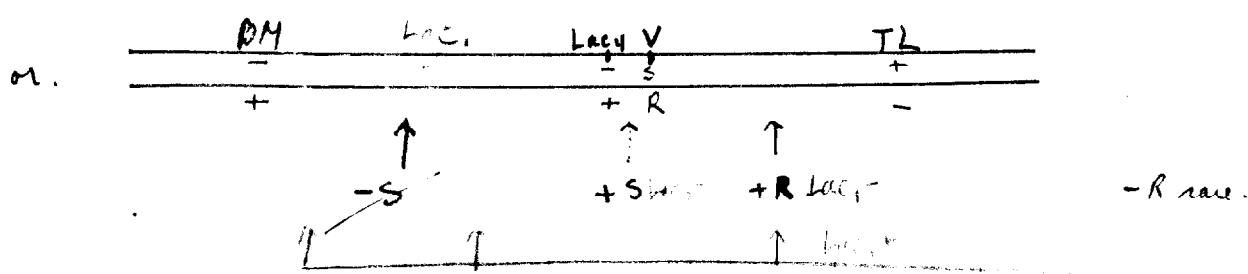
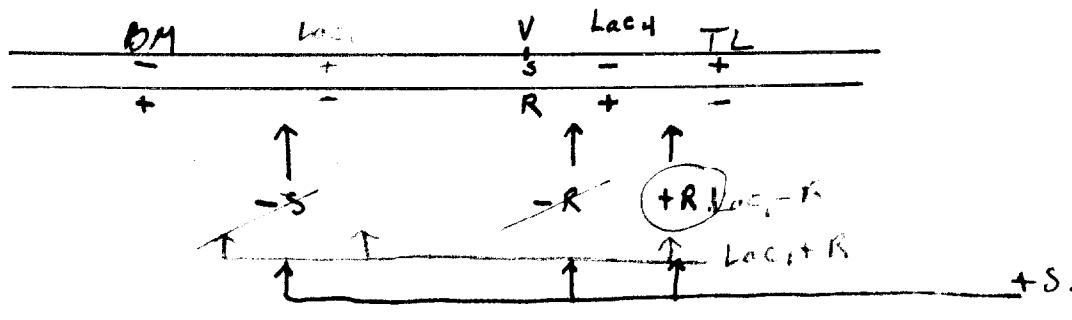
May 24, 1948.

On EMS-Lac B,

- ① W-108 × Y40. Cross n.g. W-108 checks very largely
lac+ (ca 1:4)
- ② W-67 × Y46. Only are occasional + colony. None on 3 glucose
plates.
S+ tested All V, R.

What linkage relationships are indicated if the Lac_4 - are merely not recovered? The combinations are:

BM Lac+ V, R TL. × Lac- V, S. Lac_4 may simply be closely linked to V, or situated so that a triple interchange is required to give a Lac+ V, S combination, e.g.



Crossing Media

203

May 24, 1948.

Basic salts + EMB +:

Lactose series + TLB, BM

L.

glucose series + B₁

G.

1. Na succinate 1%

2. " " .5%

3. Asparagine 1% Designate EMA. (cost > \$1/liter)!

4. " " .5%

5. Na aspartate 1%

6. " " .5%

7. Na glutamate 1%

8. " " .5%

(A). Cross W-108 x Y40 on a plate each of series G. IP24.

(W-108 is ca 1/4 Lac + ∴ ratios cannot be concerning.)

(B). streak out on a plate each of series L.

- (A) 3P.
 1+2. No. prototroph colonies. Prinpoint background. (poss. a few v.sns.)
 3.+4. Numerous prototrophs > 1 mm. diameter, many already showing
 Lac+ or -. 4/a little larger than 3, but uncertain.
 5. Prinpoints
 6. like 5
 7. Prinpoint background.
 8. 557.

Asparagine, so far, is the most superior supplement.

8:30 P.

1,2, 7,8 prinpoint background.

3,4. (asparagine) 3: v. well developed colonies, especially Lac+. Numerous - colonies not so large but more numerous.
 4: do. Lac+ more accentuated Lac- possibly slightly smaller.

5,6 (acetate). 5: Fewer colonies, Lac+ only
 6: Ditto.

9 A 26.

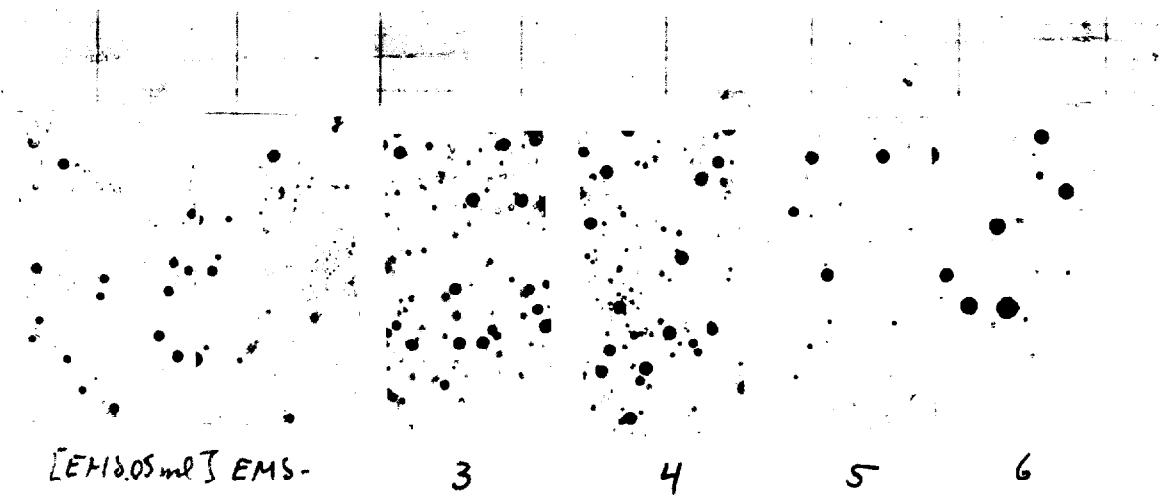
1,2, 7,8. Prinpoints, v.g.

3 shows slightly higher yield than 4, which permits less crowding on 4.
 Lac+/- character is perhaps more distinct on 4. Background is satisfactory - probably less mottled on 3.

5. Yield about $\frac{1}{5}$ of 3. Lac- tend to be smaller than Lac+, but not unsatisfactory

(. like 5. EMS. standard: Excessive background; yield poor & variable in 3 v/s 4)

203a.



Map Lec₂ + Lec₃.

204

May 26, 1948.

An Lec EMA :

① Y4/6 x W4/5

② W10/8 x W1/5

① No yield.

②.

	-	+
4	•	0
3	0	0
2	0	0
1	0	0
L	0	0
<hr/>		
12.	0	0

Yields too low.

May 27, 1948.

On lac + Glu A:

- (3) W-67 x Y46 No yield (1 colony / 4 plates.) 5+V^R. No -
(4) W-67 x Y64.

A 29:

Yield much higher in W-67 x Y46 than in W67 x Y64.

All - (many started to papillate - probably Y64).

Test on TI.

- (4): 33 - all R. No +.

~~May~~ 28, 1918

- ①. W-145 x Y40^R. Lac
- ②. W-337 x Y40^R ← Lac B.
Lac O
Lac B.
Mol B.
- ③ W-145 x Y87.
- ④ W-45 x W-145
- ⑤ W-337 x Y87.
- ⑥. W-337 x W45. Lac B.

A31:

3:

	+	-
1	9	
1	3	
0	2	3

	2	3	37.
1	1	8	

$$3+ : 53 - / 56.$$

2: L_B.

	0	0
2	0	
0	0	

$\therefore \text{Lac}_5 + \text{Lac}_1 -$

M_B (0 3 plates)

L_O

	0	0
0	0	
4	0	

L_B, 2 plates summed + and -

1:

	+	-	27 60 / 87
9	16		
8	14		
2	4		
→ 5	12		
0	5		
3	6		
0	3		

2059
cont'd

	+	-
Y:	0	2
?	0	0
O	0	1

Yield too low for satisfaction

(5) On Lac B.,

1	0
0	1
0	1
1	0
<hr/>	
2	1

Background rather heavy, but
not ruined.

(6) On Lac B.,

1?	0
1?	0

Dense background.

Many small phototographs.

Plate only satisfactory. Hole!!

(7) Colonies picked up exhaustively + tested on lac EMA + TI.

-R	-S	+R	+S.
11	4	3	1
13	4	3	4
11	3	5	0
15	3	0	1

50 14 11 6 | 81.

64 17

61 20

2059.

June 2.

(3) (W-145 x Y87)

+	-
6	25
4	29
3	4
4	20
17	78
\sum 95	

(6) W-45 x W-337.

Plates crowded. About 1/2% + colonies!

Brace repetition!

January 29, 1948.

- | | | | |
|-----|-------------------------|-----|--------------|
| (1) | W-67 x Y46 | (1) | W-67 x Y46 |
| (2) | W-67 x Y64 | (2) | W-133 x Y40. |
| (3) | W-45 x Y46 | (3) | W-67 x W-133 |
| (4) | W-133 x Y40 | | |
| (5) | W-133 x Y40. | | |

A 31.

(1) Yield ①. (Glucose EMB)
10 plates + 3 Lee plates.

(2). Ox: - > +. (linked to B4).

(3). 2 plates streak to lac B.

June 3.

2: On Lo, 14+ : 2-

on LB, many plates show more - than +. Many mucous colonies are papillate or have turned color.

On smears:

+	-
9	12
14	18

$$\begin{array}{r} \\ \hline 31 & 45 & | 76. \end{array} \quad \chi^2 = 10.9 \quad p = .001$$

X

2 - strayed to Lae B.A., T₁.

-R -S +R +S

L₀ plate 10 3 0 2"+" of previous page ~~was~~ may not be truly so.L_{B₁}.

$$\begin{array}{r}
 16 & 12 & 10 & 0 \\
 13 & 8 & 2 & 0 \\
 \hline
 29 & 20 & 12 & 0 \\
 \\
 \hline
 & & 49 & 12
 \end{array}$$

These plates are truly to offer accurate study.

3: 13 all -

May 29, 1948.

Irradiate suspensions of S-20, and 21 as follows.

Grown (6 h.) suspensions of S--- in YZ-glucose, shaken, resuspended in H₂O.

S-20 exposed to Hanovia output at aperture of lamp in quartz flask shaken by hand.
5 ml. suspension added, .5 ml removed at stated intervals to 10 ml. tubes of
YZ glucose shaken at 37.

S-21 exposed in 1 ml. lots in 10 cm Petri Plates, exposed at table level (ca 12 cm)
.5 ml samples removed from each plate.

— S-20: 10, 30, 60, 120 and 180 secs. Samples 10+30+++ 60+ ++.

S-21 2, 5, 10, 20, 30 and 60 secs. Samples 2-10 +++ 20-60 +++.

Dilute S-20, 10 second and S-21 5 second exposures 10⁻⁷ and
plate in minimal layered agar, 2 P 30.

For reference, S-20 = SW-1 and S-21 = SW-2.

Ca 30 plates each, and 10,000 colonies.

11 picked, 9 grew up in series S-20-21

23 " , 21 " " series S-20.

Numbers 1-21 are S-20; 22-30 are S-21.

Mutants SW-3 and SW-4 () from S-20

SW-5-8 () from S-21.

Test putative *Salmonella* mutants.

208 -

T/10) HC V,ts Y.Ex. ^{Lac}_{EMB} ^{lact}_{EMB}.

1	++						
2	++						
3	++						
4	++						
5	++						
6	++						
7	+	++	- +		+		
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19	- +	++	- +	++			sw-3
20							
21							
22	-	-	-	++			sw-4
23	-	-	-	-			sw-5
24	-	-	-	++			sw-6 S.O. or glucose
25	-	++	-	++			sw-7 agar.
26							
27							
28							
29	-	++	-	++			sw-8
30							

All - and third typical except 24 which is thin with - flagella

All + and typical exc.
24 which is thin.

is derived
AA:

	①lysine	②methionine	③dlec, col,	④phosphatase	⑤cit. thym.	⑥arginine	O	HC	A.A.
sw-3	+	+	±	+	++	±	±	+++	++
4	±	±	±	+	++	±	±	+++	++
7	-	-	+++	-	-	-	-	++	++
8	-	-	-	++	-	-	-	++	++
S-21									
	YNA	Y.Earth.	N2ase	Pur+Pyr	H-C.+U,ts.	O			
5	-	++++	-	-	-	-	-		
6.	-	±	+	-	++				
S-21.	++	++	++	+	++	++		U,ts. N2ase	Y.

(deficient)

Plate a mixture of W-108 and T1 on Lac EMB. Select 8 surviving colonies and streak out 3 times. Test these 8 and W-108 on T1 and on T5:

	T1	T5	
1	R	R	W-399
2	R	S	W-400
3	R	R	
4	R	R	
5	R	R	
6	R	S	
7	R	R	
8	R	R.	
W-108	S	S	

The R,R types are presumably V_1^R and the R,S V_{1a}^R . Select 1 and 2.

This is an unusual preponderance of V_{1a}^R : (2/8).

1

See 23/c. W-400 is $T5^R$.

Lac_{2,3,4,5} and *V₁, V₄*^R. Crosses. (209) 210

May 31, 1948.

On Lac + Glu EMA¹.

- (1) 58-161 x W399
- (2) 58-161 x W400
- (3) W45 x W399
- (4) W45 x W400
- (5) W67 x Y10
- (6) W67 x Y64
- (7) W67 x Y46
- (8) W145 x ~~58-161~~ 58-161.
- (9) ~~W145 x 58-161~~
- (10) W145 x W45

Jun. 3.

1. Color faded. Pick colonies at random for test in Lac, T1.
2. ditto.
3. No yield (1 col / 3 plates).
4. All - ¹³⁸I colonies, probably not faded. Close linkage of Lac₂ to Lac₃ confirmed.
Sensitive to Maltose. All out of 53 are Mal- with heavy + contamination.
5. Yield OK. > on glucose than on lactose. Perhaps 1 or 2% of colonies on Lac are -.
6. Tiny colonies just starting
7. No yield, on glucose or on lactose
8. Like 1. Pick at random to lactose.
9. 1 plate. 1+, 3 or 4 - colonies.

210 - 1

①. 2 classes of colonies. large spreading, probably +
and small compact, -?

Frequencies: "+ " - " | 598. Ca. 3.3% + (in agreement with
previous observation)
Test "+" and "-" separately on EMA-hacB.

	-R	+R	-S	+S	
""+	27	0	0	0	
"-"	53	0	1	0	
	80	0	1	0	81

②. Same as ① in appearance & proportion of +.

"+"	30	0	0	1	
"-"	53	0	0	0	
	83	0	0	1	84

Altogether only about 2 / #8165 or ca. 1.5%

59. Pick from gluEMB to lactose EMB.

53 picked. 4 lac-. Strains out on LacEMB.

210 - 59 (1-4).

Nutrition of W-67.

217

May 25, 1948.

Test on: (ell + BM) P25 - A26.

(1) T(m) + 1% succinate

(2) " " + Y. ex.

(3) " " N2Case

(4) V, ts.

W-67 is not nutritionally
distinguishable from 58-161.

T(0) [glucose-asparagine].

(11) —

(12) Y. ex.

(13) N2Case.

(14) V, ts.

	A W-67	B 58-161.
1	± ++	± ++
2	+	++
3	+++	+++
11	+++	++
12	+++	++++
13	+++	+++
4	-	-
14.	+++	+++

Streaks out on Lac A + BM etc. P26
11A. A27 Lac A. Glu A. Lac E MB.
purple. ++ v. small

11B. +++ +++ +++
Lac Y - should be produced without
influence on Glu A plates

P26 + A27.

May 30, 1948.

Incubate + shake in Y2-glucose tubes, overnight,
① ⑤ ③.

Bs 16, Bs 164 \times and Marburg = Bs+.

Wash + resuspend cells in = vol. ~~and~~ citrate saline buffer.

Spread 1 drop each of ① + ② together and separately on T(0) plates. Also mix Y2 with .5 ml inocula together + separately + shake. Also carry along ③.

June 2, 1948.

① 1 colony, 1 slight background

② 0, 0. Practically no background.

(1+2). 11, 6 background rather heavier than with ① only.

(And other numbers).

Also plate suspensions from above:

Read A/V:

① 0, 0

② 0, 1

① + ② (inc. separately) 4, 9. } (odd!)

(1+2. inc. together). 1, 0.

The possibility of recombination is not ruled out by these experiments.

Drug resistant mutants of *B. subtilis*

June 2, 1948.

P. Spread .1 ml of suspensions of p. 212 on Nutrient Agar plates containing indicated u/ml of penicillin + streptomycin:

① Bs 16 (tryptophanase) ② Bs 164x (lysineless).

①. P1. Scattered colonies in thicker portions of plate

P5 ca 20 colonies distinct; some smearing confuses count
P10 5 distinct colonies.

S1 Almost confluent background, with papillae

S5 ca 200 distinct colonies, no background

S10. ca 100 distinct colonies " "

N.A. Heavy smear.

②. P1. ca 12 distinct, v. large colonies (smearing).

P5. 2 colonies, quite large

P10. No colonies.

S1. As ①.

S5. (Plate rather dried). Ca. 500 colonies (counted?).

S10. Several hundred colonies.

NA Heavy smear.

Keep highest plates for purification on N.H. $\bar{+}$ + $\bar{5}$ drug.

Streak out. Test ⁵ single colonies on P10, S10 and NA.

	NA	P10	S10
16/P10	++++	-	-
16/S10	++++	-	++++
164/P10	++++	-	-
164/S10	++++	-	++++

very sharp destruction
on streptomycin agar.

P3. Add Y2-glucose = P10 and S10 to obtain cultures for higher step mutants. A4 Spread 1 drop each culture on NA = Read A5.

16/P10 P5 P10 P50 P100 S10 S50 S100 S500
 v. numerous v. numerous v.n.s. Large 1-200 Large 20 small. 0 later 6-10 small. 0

K
 See above
 not resistant

16/S10 almost scattered. ca 100 scattered colonies. 30 distinct small. ca 100 scattered colonies. (ca 100, scattered colonies.)

164x/P10 100 200 20-30 0 1-200 2 large 15 small 0 0
2.

164x/S10. numerous sm. 500 200 100+ small scattered 40 6 0
 colonies. colonies small cols. colonies.
 almost (small).
 small.

Test the following, as indicated.

5500 S100 P10 P100 S10

16 S10
 16 S10/S100
 16 S10/P100

See next page.

Test colonies from the following plates & cultures.

	P10	P100	S10	S100	S500
" 16S10 "	S	S	R	R	S
" 16P10 "	S	S	S ^R	S	S
" 164S10 "	S	S	R	S	S
" 164P10 "	S	S	S	S	S
16S10/S5001	S	S	R	R	R ^S
164S10/S100 ²	S	S	R	R	R ^S
16. P100 ³	S	S	S	S	S
164. P100 ⁴	S	S	S	S	S
16.S10.P100 ⁵	S	S	R	R ^S	S
164.S10.P100	S	S	R	S	S

Streptomycin resists are OK, sharp distinction between the 10 and 500 unit levels. No penicillin resists so far noted.

Standout, on NA, the cultures 213B-1 and 213B-2

June 3, 1948.

(1) W-337 x W-45

(2) W-145 x Y40

(3) W-126 x Y40.

Simultaneously, streak out W-45 and Y40 on Lac A + (B + I).

P4. W-45 + Y40 are well grown on the synthetic medium; but none of the cross plates show any colonies of significant size.

P5. 1: No colonies on Lac A + B.,

2: No colonies on Lac A.

Some plates of T(B₁) have colonies, irregularly scattered

3: No colonies on T(B₁) or Lac A + B.¹

P6. 1. No colonies.

2. Few colonies from T(B₁) to Lac T1.

3. 1 + colony on / plate.

June 4, 1948

W-133 x 1/10. on

A) T(B.)

B) Lac A(0)

C) Lac A(B.)

D) Lac A(B.)

P6. Colonies appearing on D, after on C. Ca 6/plate on A.
p8.

A. ca 6/plate

B. 2+ / 5 plates

C. ca 100/plate 1:1 + : - (Heavy background.) 59+:51-

D. ca 50/plate 26+:16 -

A. Put to water + test suspensions
on T/ on Lac EMB. - Background too heavy
All lac+ v R.

B. —

C. & D. pick + and -
separately.

	R	S
+	24	1
+		
=	20	6
=		
	45.	7

	R	S
+	17	0
+		
=	11	1
=		
	28	1



216

Salmonella Irradiation
double mutants.
, "crosses".

June 4, 1948,

Irradiate washed 8 h. suspensions of SW-3, SW-7, SW-8 and S-21, in 1 ml. lots in open Petri plates. Recover $\frac{1}{2}$ ml samples to NZ-glucose broth, and shake overnight. In S-21 series, plate .05 ml sample from the initially inoculated cultures to estimate killing rate. 5, 10, 20, and 30 seconds under Hanovia lamp.

Assuming inoculum of $.5 \times 2 \times 10^9 \times 0.05 = \underline{\underline{5 \times 10^6}}$, the killing can be estimated.

S.	S.
secs.	5000 ^{ca.}
10	239
20	8
30.	10.

These suspensions were inadvertently autoclaved.

- 21

- Irradiate the above washed suspensions, as above, dilute as indicated and plate directly into detection plates. SW-3 suspensions not available
- | | |
|--------|---|
| - S-1 | 10 sec.,
10P6, 36L. Cover in NZ Case-Tryptic extract - Agar. |
| - SW7 | SW7 series not yet grown. Do not cover. |
| = SW8. | |

Mix on T(0) plates single drops of SW-3, -7, & -8 as indicated.

- | | |
|------|--|
| 3 | colonies P6. |
| 7 | 3, 2 |
| 8 | 2, 1 0 (+ certain.) , 0. P7 ca. 50. |
| 3x7 | 0. 0. other plate heavily cont. = Aspergillus. |
| 3x8 | 2, 1 Numerous plaques noted (lysozyme?) |
| 7x8. | 2, 1 mainly with 1 or 2 colonies. See 217. |

SW7 series formed small colonies only on June 9. Threw out plates. L-L-V supplement is obviously not optimal in the proportions used.

SW1 and SW8 series. Almost 20% of SW1 and 10% of SW8 are small colonies. Either nutrient or contaminant. Picks + test about 100 in each set. Picks colonies to sm. tubes 1/2. with loop, streak on EMBAce and put residual inoculum from loop into T(0) + tryptophane. Most were - in small tubes; the following were +:

SW1: 19, 29, 39, 59, 79, 89, 99, 100.

9th row tubes were more elevated. Could this account for some of them? (Heavier aeration?).

SW8. (delay scoring).

Test SW1: 1-3 and SW8 1-2 on T(T₂) large tubes.

All +++.

Small tube tests are inaccurate. T.O. expt.

217. Plate SW-3 + SW-7 on N.A. in 10⁻¹ dilutions individual.

SW-3 SW-7

10⁻¹

10⁻¹

~~conf.~~ confl. growth

10⁻²

10⁻²

isolated colonies (ca 1000)

10⁻²

10⁻²

"do."

10⁻³

10⁻³

confluent growth. No plaques-

10⁻⁴

10⁻⁴

No evidence
of lysis/plaques
on nutrient agar.

June 5, 1948.

sw-6. (pab.)

	O	Vits.	pab.	HC	pab+HC	pab, HC, PP.
--	---	-------	------	----	--------	--------------

after 18-24h.	-	+	+	-	++	++
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sw-7 (leuc, val, val).
S. Typh. control.

-7	O	HC	L	IL	V	L·IL	L·V	IL·V	L·IL·V
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leucine - isoleucine - valine

+ (Y)	-7	+++	+++	+++	+++	+++	+++	+++	+++
-------	----	-----	-----	-----	-----	-----	-----	-----	-----

sw-8. (trypt.)

O	trypt.	indole	anthran.	nicotinic
---	--------	--------	----------	-----------

18h.	-	+++	++	-	-
------	---	-----	----	---	---

later

+++

Medium for crosses.,
 Lac₁, Lac₃. Effect of sheltering on crosses.

June 8, 1948.

Grow Y53, Y40 + W108 in Y2... (glucose or glucosine)

A-shelter B-unsheltered. Mix = volumes + plate

1 drop each on Lac A + B, and Lac S + B, etc., T(B₁).

1. Y53 × Y40

$\left. \begin{array}{l} B \text{ suspensions are, of course, much less} \\ \text{dense than } A. \end{array} \right]$

2. W108 × Y40.

A10.

1A. 27, 23, 34 on T(B₁).

(2-1+), 0. on Lac A.

7, 4, 11 tiny colonies on Lac S.

1B. 1, 0 on T(B₁).

>100, ~~—~~, Lac A. ex sp. medium colonies.

16+, 22-, 15+ 24-, Lac S. Better definition of +/- but not yet quite ready.

2A. 5 on T(B₁).

4, 5, 7 on Lac A. All -

4, 4 on Lac A -

2B. 52-2+,

Lac A. } +/- definitely good, somewhat
Lac A. } better than on S.

97- 6+

30- 1+

Lac S. } Conclude: Sheltering is certainly
} deleterious to crosses!

21. 3+

P II.

1B. (Lac S.)

34+ : 31-
[Too many + J.]

Lac A.

9+ : 15-

2B.



1B-S

2B-1

To 10 ml T(0) add:
18h.

A. 0.2 mg dl-isoleucine and:

1	valine	0	±
2		.02	.
3		.05	.
4		.10	.
5		.20	±

B. 0.2 mg. dl-valine and

1	10	isoleucine	-
2	.02		±
3	.05		±
4	.10		++
5.	.20.		+

(Try adding leucine to this!)

Cf. 70:30 used by Bonner et al. for 16117.

C. ~~1/2 dl - Isoleucine
1/25 dl - Valine~~

~~.12 dl - isoleucine
.02 l - leucine
.03 dl - valine.~~

(optimal for Neurospora).

D. H.C. ++.

Ca 2:1 valine: isoleucine

is best so far.

sw-5. Tween 80, yeast RNA, Oleic acid, Cognosanase All -
4. Arts. ++

Phenolphthalein Phosphate

221

Prepare plates of NA to which Na Phenolphthalein Phosphate (Paul-Lewis; sterile filtered) is added.

Streak out A. (SW-7) B. (K-12) & C (*B. subtilis* 16).

After 24 hours growth, expose plates to NH_3 vapor.

A. + B. show no change in color at any conc.

C: 100 μ No sharp change

300 μ colonies became light pink

1mg. colonies became a dirty pink.

Also:

	SW 3	SW 7	
Dulcitol	v. weak +	v. weak +	
Rhamnose	++	-	
Cellobiose	- alk.	-	
Salicin	- *	- *	blue tinge to colonies not hitherto noted
Mositol	-	- pap. (S.V.)	

Note: very weak + fermenter of
Mannose & of Mositol can be
secured by selecting papillae of SW 7.
These are extremely weak.

"

Reactions.

June 10, 1948.

Irradiate SW-7 and -8, 1 ml in open petri dish, 10 secs.
dilute 1 ml/10 broth, and ~~add~~ spread 1 drop per plate
of xylose + arabinose EMB.

- ① SW-7 on arabinose; SW-8 on xylose.
- ② Also, about 10 plates each., 1 drop whole culture spread on plate and irradiated directly, 5 secs.
SW-3 / arabinose SW-7 / xylose.

16h. SW-7 and SW-8 are xylose-negative, to surprise!

SW-7 treatment on arabinose was excessive & only a few dozen colonies per plate. No mutants.

Suggests selecting for Xyl + mutants! after 3 days. becomes easily isolated & purified.

Check fermentation reactions on -EMB:

SW-3

Xyl	++ ✓
Ara	++ ✓
Glu	+++ ✓
Kal	++ ✓
Gma	++ ✓
Mal-	++ ✓
Sorb	+ +± -
Mannitol	++ ✓

SW-7

- ✓	<u>lysine and xylose??</u>
++ -	
++ -	<u>Salmonella fermentatio</u>
++ ✓	acid much slower than
- ^{coli!!}	<u>in other part of plate; + clumps</u>
++ ✓	<u>need to be cleared.</u>
+ +±	<u>is + except in crowded areas.</u>
++ -	

Tetragolium Reagent.

June 11, 1948.

Incorporate 50 v/ml T2 Reagent into agar + 1% lactose as indicated.

- A. N2 Broth ($\text{PO}_4^{\text{2-}}$ buffer) = N2L
- B. " + 1% Na formate N2LF
- C. Nutrient broth NBL
- D. " " + Formate NBLF.

A. II. Stake out, on each plate:

$$K = K-12$$

$$S = B. subtilis 16$$

distributed on each plate.

$$SW = SW-7$$

$$W = W-400 (\text{lac}_2^-).$$

A. K: colonies colorless or faint pink. 1 large dark red colony (223-1) → (223-2)

SW isolated colonies dark red.

W: colonies dark red.

B. As A. K more to red but not intense.

SW red & white colonies in the colorless zone.

W all colonies dark red; definition somewhat better than A.

C. K nearly colorless; All colonies of W & SW show up very well.

D. About the same as C. K more pink. S + SW somewhat more intense.

Test 223-1 + -2 on homologous media for lac-E-MB.

1 is lac- 2 is lac+ (probably colony from SW-7).

See over:

Mix K, + W and streak on NL, EMB Lac

+ and - easily scored in each other's presence provided the plate is not too crowded, when one finds the - 'score' as colorless. The method shows considerable promise for the detection of non-fermenters.

Different bases should be tried in attempt to obtain uniform coloration of the - , even in crowded areas, which would facilitate their detection.

SW-5 June 11, 1948.

	Y. Ctx.	$\approx L.$ Bulgarian factor.	
1.	5 mg	+++	=
2.	1 mg	+	=
3.	500 Y	\pm later +++ (nw).	=
4.	100 Y	-	=
5.	20 Y	-	=

} not *L. Bulgarianis* factor.

SW-7. Valine 0.2 mg/tube.

Isoleucine

1. 1.0

2. 1.2

3. 1.4

4. 1.6

5. 1.8

6. 2.0

~~7. Ditto + .2 mg l-leucine.~~

11

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13

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Salmonella phage.

June 14, 1948.

Cultivate S-20 + S-21 in 1/2 overnight, i shaking:

Centrifuge raw Madison sewage & filter supernatant. (Sewage Filterate)
Add 1 ml SF + .5 ml S-20 or S-21 to 10 ml broth.

Incubate 6-8 hours. Both are thoroughly turbid cultures.

(225-20, -21). Sediment bacteria! Test supernatant for
phage by ① 1 drop "phage" + 1 drop bacteria ② streak
out phage & bacterial smear.

225-20: ① } large plaques noted in both. (May correspond to the
phage attacking resistant bacteria?) - small plaque
② } phage also noted.

225-21 ① pattern of resistant colonies.

② small plaque phage noted along streak.

suspend plaques in water and streak out on homologous bacterial
smears. [Crude phage suspension should be filtered].

After several streakings, pick from single plaques to
broth cultures + recover phages. These may not be pure.

Sp-1 S20 ^{small} ~~large~~ plaque

Sp-2 S20 small "

Sp-3 S21 small "